received 3 times per week 25 IMJ of 0,2 ml isotonic solution, *CS group* - received 3 times per week 25 IMJ of 0,2 ml 10% solution CS. After euthanasia tissues were fixed for 24 hours in 10% neutral buffered formaldehyde. Kidney, heart and liver were investigated by histological and histochemical methods. The material was studied by blind method.

**Results.** At the end of the experiment only 5 males survived in placebo group, in the CS group - 15males and 10 females. The alive animals were decapitated. In placebo group fibrocartilage articular surface was destroyed and remodelled. Tide mark was absent. Acid glycosaminoglycanes (GAG) depletion and collagen network fragmentation have been estimated. Severe vasculitis in all organs were found. There were severe nephritis, myocarditis and hepatitis too. In the CS group cartilage was uniform, with normal thickness and layer differentiation. Cartilage reparation processes have been estimated as follows - the number of double-nuclear chondrocytes increased and accumulated in groups. GAG increased too. Collagen structure re-established. Tide mark was safe. Dystrophy and mild vasculitis in heart and liver, severe alteration in kidney were found. There was significant difference between the group results ($p<0,05$).

**Conclusion**. Not only cartilage, but organic lesions, such as vasculitis, nephritis, myocarditis and hepatitis are influenced after chondromodulators' treatment of MRL/l mice. These results provide some insight into the anti-inflammatory mechanism of CS action.

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## FOLLISTATIN ALLEVIATES SYNOVITIS AND ARTICULAR CARTILAGE DEGRADATION INDUCED BY CARRAGEENAN IN MICE

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**Purpose:** Osteoarthritis (OA), a chronic degenerative joint disorder characterized by articular cartilage destruction and osteophyte formation, is prevalent in our society as a major cause of disability. Molecular pathogenesis of OA is not fully understood, it is considered that acute joint inflammation plays significant roles in the onset of OA. This idea is supported by various animal experiments, since articular cartilage degradation is induced by the intra-articular injection of carrageenan or zymosan. Previously we showed that intra-articular injection of recombinant human BMP7 (rhBMP7) inhibited cartilage degradation induced by zymosan partly through the inhibition of inflammatory cytokine expression such as IL1-beta in the joint. From these data we hypothesized that TGF-βeta/BMP signal maintains joint homeostasis by controlling inflammatory status. Here we report that follistatin, an endogenous inhibitor for Activin which belongs to TGF-beta/BMP family and works as a proinflammatory cytokine, effectively alleviates synovitis and articular cartilage degradation induced by the intra-articular injection of carrageenan in mice.

**Methods:** This study was approved and conducted in accordance with the guideline of the animal committee of Tokyo Medical and Dental University. Male C57Bl/6J mice (12weeks old) were purchased from ORIENTAL YEAST co.,Ltd (Tokyo, Japan). They were housed under a 12-h light-dark cycle and allowed food and water ad libitum. Twelve mice were randomly divided into two groups (n=6/group). Mice were anesthetized by the inhalation of 5% isoflurane in oxygen. Under deep anesthesia, a solution of 30μg lamda-carrageenan (Sigma-Aldrich) in 5μL saline was injected into the left knee joint through the lateral margin of the patella tendon. Recombinant mouse follistatin (25ng in 5μl in physiological saline, Sigma-Aldrich) was injected into the left knee at 30 minutes before carrageenan challenge. Mice were maintained in cage ad libitum for 3 days after the challenge. Knee joints were dissected, fixed in 4% paraformaldehyde, decalcified, embedded, and 5μm sagittal sections were prepared for histology. Integrity of articular cartilage and synovium was assessed by Hematoxylin and Eosin staining. To assess articular cartilage damage, three sections (apart from 150μm respectively) were stained by Safranin O and 400 μm in width of articular cartilage between anterior and posterior edge of medial meniscus was contoured into 3 areas according to the dyeability: Grade I; intact cartilage, Grade II; mildly denatured cartilage with reduced safranin O staining, and Grade III; severely denatured cartilage with no Safranin O staining. Each area was measured using Zeiss Axio Vision Image Analysis system. Kruskal-Wallis test followed by Tukey-Kramer methods was used for statistical analysis.

**Results:** Image analyses indicated that dyeability of articular cartilage by Safranin O was significantly reduced by the single intra-articular injection of carrageenan at 3 days (Grade I: 21.4%, Grade II: 33.8%, Grade III: 44.8%), although we did not observe any obvious alteration in the articular surface structure at this stage. In contrast, the loss of dyeability after carrageenan injection was significantly improved by the pre-injection of follistatin (Grade I: 26.4%, Grade II: 73.6%, Grade III: 0%, $p<0.05$). Hematoxylin and Eosin staining showed that the cellularity of synovial tissue is greatly increased in carrageenan-injected mice. In contrast, these inflammatory responses were greatly alleviated by the pre-injection of follistatin.

**Conclusions:** Carrageenan-induced arthritis is a well-established experimental model to investigate inflammation-mediated articular cartilage degradation in rodents. Here we report that follistatin effectively inhibits the loss of Safranin O dyeability of articular cartilage induced by carrageenan. Our data strongly suggest that the Activin signal pathway is involved in the process of joint inflammation and proteoglycan loss in the cartilage matrix. We believe that our experimental system will be of great use for analyzing the molecular events undergoing the onset of OA.

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## NEW EVIDENCE LINKING THE IN VIVO IMPLICATION OF 4-HYDROXYNONENAL IN THE OSTEOARTHRITIS PATHOGENESIS.

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**Purpose:** To demonstrate the *in vivo* involvement of 4-hydroxynonenal (HNE), a major aldehyde derived from lipid peroxidation of n-6 polyunsaturated fatty acids, in the osteoarthritis (OA) pathogenesis.

**Methods:** Protocol 1 - OA was induced by anterior cruciate ligament transection of the right knee in crossbred dogs. There were two experimental groups (n=4 dogs/group): placebo and carnosine (an HNE-trapping drug, 50 mg/kg/day) given orally for 8 weeks Protocol 2 - Vehicle or pathophysiological dose of HNE (100 μM) were injected weekly into the right knee joint of crossbred dogs (n=4 dogs/group) for the entire duration of the study (16 weeks). We conducted macroscopic and histomorphological analyses of cartilage of the femoral condyles and/or tibial plateaus. We also conducted immunohistochemical analyses in cartilage explants for the following antigens: HNE, agrecannase-2, and matrix metalloproteinase -13 (MMP-13).

**Results:** In the protocol 1, treatment with carnosine reduced the severity and histopathological score of OA cartilage lesions as well as the levels of HNE, MMP-13 and agrecannase-2 in cartilage explants. In the protocol 2, the intraarticular injection of HNE induced cartilage lesions on the tibial plateaus and femoral condyles with prominent osteophytes on lateral condyles. The expression of both MMP-13 and agrecannase-2 increased in cartilage explants from HNE-treated dogs.

**Conclusions:** This is the first in vivo study to demonstrate the pathophysiological role of HNE in OA. The fact that carnosine abolishes HNE production and a number of factors known to be involved in OA pathogenesis renders it a clinically valuable agent in the prevention of this disease.

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## A COLLAGEN-PLATELET COMPOSITE TO STIMULATE HEALING AFTER ACL SURGERY ALSO MINIMIZES CARTILAGE DAMAGE IN THE ACL INJURED KNEE

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**Purpose:** ACL injury is a risk factor for early post-traumatic osteoarthritis (PTOA), and the gold standard of treatment, ACL reconstruction, does not reduce this risk. The mechanism of PTOA in the ACL injured joint is likely due both to the initial inflammation and ongoing subtle mechanical instability. Recently, intra-articular implantation of a collagen-platelet composite (CPC) during surgery in animal models has been shown to improve healing following bio-enhanced ACL repair or ACL reconstruction procedures, though the impact of the CPC on articular cartilage remains unknown. We hypothesize that cartilage integrity following bio-enhanced